THE OCCURRENCE OF DINOFLAGELLATE LUMINESCENCE AT WOODS HOLE[1]

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The use of sensitive photomultiplier radiometers has brought about recognition of the very widespread occurrence of bioluminescence in the oceans. Flashing has been recorded in nearly all parts of the oceans and in almost all locations studied (Clarke and Wertheim, 1956; Clarke and Kelly, 1965). At night using proper equipment luminescence may be recorded continuously in the upper layers of the ocean, usually within the euphotic zone and above the thermocline (Clarke and Kelly, 1965; Yentsch, Backus and Wing, 1964). However, the organisms responsible for the near-surface luminescence have been definitely identified only in exceptional circumstances such as during unialgal blooms (Harvey, 1952) or in very eutrophic embayments such as Phosphorescent Bay in Puerto Rico (Clarke and Breslau, 1960; Seliger *et al.*, 1962).

Many authors have suggested but not shown that the organisms responsible for this common near-surface luminescence are the dinoflagellates, very common phytoplankters with carbohydrate cell walls and two flagella, one encircling the cell at the midline and the other directed posteriorly (Clarke and Kelly, 1965; Harvey, 1952; Kelly and Katona, 1966; Schiller, 1937). The work reported here is one of three studies conducted under diverse conditions and at diverse locations to determine the relationship between the occurrence of luminescent dinoflagellates and the common oceanic bioluminescence.

In the work described here the dinoflagellates and bioluminescence present in Woods Hole Harbor between November, 1965, and November, 1966, were studied. The species capable of luminescence were identified, their concentrations in the water were measured throughout the year, and the amounts of luminescence in the water were determined at least once a week by testing samples brought into the laboratory darkroom. The populations were also tested for susceptibility to light inhibition of luminescence and for the presence of an endogenous diurnal control of the amount of luminescence produced.

In a preliminary study made during August and September, 1965 (Kelly and Katona, 1966), water was brought into the laboratory at Woods Hole Oceanographic Institution and the luminescence in the water was measured. The species of armored dinoflagellates capable of luminescence were identified and although cell concentrations were not measured, these organisms appeared to be present in sufficient numbers to explain the luminescence in the water. In addition, the popula-

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tions displayed an endogenous rhythm in luminescence and in sensitivity to light inhibition of luminescence. It became apparent that a study over an extended period of time at one location was required to show if luminescent dinoflagellates are usually present in sufficient numbers to explain the amounts of luminescence recorded in the water, and whether endogenous diurnal rhythms of luminescence are present throughout the year.

Luminescence of Isolated Cells

Individual dinoflagellates were tested to determine which species were capable of contributing to the luminescence recorded in whole water samples. Cells were isolated by micropipette into 0.5 ml. of water and stimulated in front of a photo-

Table I

Dinoflagellates examined for flashing. Numbers refer to months in which tested, from 8, 65 to 8, 66. One to ten specimens were tested on each date given

Species	Luminescent		Fluorescent		Photosynthetic pigments
	yes	no	yes	no	
Ceratium fusus	8, 65; 9; 10				always
C. lineatum		9; 10; 11; 12; 1		12	always
C. tripos		9; 10; 11			always
Glenodinium lenticula		9; 10; 11; 3			never
Gonyaulax digitale	9; 8, 66				always
G. spinifera	8, 65				always
Gymnodinium splendens		10			always
Peridinium spp.	varies				varies
P. claudicans	8; 10	12; 8, 66		12; 8, 66	never
P. conicum	6; 10		9, 66		never
P. depressum	4				never
P. divergens	11		12		never
P. globulus	10; 11				never
P. granii					?
P. leonis	9; 10; 11; 8, 66				never
P. mite		8, 65			?
P. oceanicum	9; 10				never
P. oblongum		8, 66		8, 66	never
P. pallidum	9; 12		12; 8, 66		never
P. pellucidum		4			never
P. pentagonum	8, 66		8, 66		never
P. punctulatum	9; 8, 66		8, 66		never
P. subinerme	11; 12; 1; 2; 3		2		never

multiplier radiometer. Methods were described in detail by Kelly and Katona (1966). The results are given in Table I. Kelly and Katona (1966) and Sweeney (1963) listed other dinoflagellate species known to be luminescent. All species showed similar intensity (approximately 10^{-4} μW per cm.^{2} at 10 cm. from the sensing surface) and duration of flashing. Occasionally a prolonged flash was recorded, lasting as long as 3–4 seconds, and the specimen could never be recovered afterward, probably because it splashed against and adhered to the side of the

sample tube. Usually, however the flash duration did not exceed 10 milliseconds. Specimens of *Peridinium subinerme* and *Peridinium pallidum* remained alive and capable of flashing for as long as 7 to 21 days while isolated in the dark in test tubes without any food other than what little might be in the 0.5-ml. Millipore-filtered sea water that contained them. None of the cells divided, although culture medium and microalgal suspensions were added to several of the samples.

Some of the species were tested for UV fluorescence under an American Optical Co. fluorescence microscope (see Table I). Long-wavelength UV produced only the typical ruby-colored fluorescence of chlorophyll-*a* (in diatoms and *Ceratium lineatum,* the only photosynthetic dinoflagellate examined). Short-wavelength UV produced a continuous blue-green fluorescence in all the luminescent dinoflagellates examined. Fluorescence originated in cytoplasmic particles ranging in size from less than 0.001 mm. to 0.003 mm. Nine species (3 or more specimens of each) were examined in this way (see Table I). All specimens of the 6 luminescent species showed the blue-green fluorescence while the 3 non-luminescent species did not. The long duration of the fluorescence indicated that the organisms were not merely stimulated to luminesce by the UV irradiation.

The fluorescence passed through a blue-green 480 nM. wavelength interference filter without appreciable visible attenuation but was attenuated by 470 and 490 nM. filters. The fluorescence therefore peaks quite sharply at or near 480 nM., which is the luminescence peak for several species. Eckert (1966) reported the "microsources" of luminescence in *Noctiluca scintillans* fluoresced in this range.

It seems likely that the blue-green fluorescence is an indication of luminescent capability. The peak at 480 nM. suggests that the fluorescence is due to the chemical that is the source of luminescence. This is not surprising since the luminescent compound must be easily excited to an active state and it should be expected that light output from this excited molecule would have similar spectral composition whether the excited state is produced by an enzymatic reaction or by UV irradiation. It also seems that luminescence probably originates in the fluorescent cytoplasmic particles, as it does in *Noctiluca scintillans.* Several other organisms such as copepods (Clarke *et al.,* 1962) and *Cypridina* (Harvey, 1952) have fluorescent luminescent organs.

The bioluminescence of several dinoflagellate species merits further discussion: *Ceratium fusus* is notable for being the only species of *Ceratium* that has been definitely shown to be luminescent (Sweeney, 1963; Kelly and Katona, 1966). Nordli (1957) maintained luminescent cultures of this species, but they were lost and he was unable to culture luminescent forms again. In the present work few of the cells tested were luminescent. This suggests that only certain cells are luminescent and that luminescence is an inherited characteristic. An inheritable ability for luminescence is also suggested by the existence of both luminescent and non-luminescent clonal strains of *Noctiluca scintillans. Ceratium tripos* has been reported by several authors to be luminescent (Harvey, 1952) but it was not so in Woods Hole and Nordli's (1957) cultures of this species were not luminescent. This places the earlier reports in doubt, although there is the possibility that both luminescent and non-luminescent forms exist as in *Ceratium fusus.* The luminescence of *Peridinium claudicans* was also variable; it was luminescent during certain months and not others in Woods Hole. The taxonomy of *P. claudicans,* however, is confused and more than one species may have been included under this name.

TABLE II

Summary of flashing rates (flashes per 30 seconds) and luminescent cell concentrations (cells per liter) during 1965–1966 at Woods Hole

	Dec.				Jan.				Feb.			Mar.				Apr.
	7	15	21	27	3	12	25	31	8	15	23	2	16	23	29	6
1. Mean	160	76	100	76	84	111	190	223	117	189	110	176	144	149	146	152
2. Maximum	179	94	128	94	109	186	208	241	159	238	181	195	198	162	165	185
3. Minimum	141	56	66	65	61	163	200	81	138	74	160	58	137	130	121	144
4. Mean, 1100–1400 hrs.	169	74	117	77	97	76	177	221	99	164	96	189	—	143	146	134
5. Mean, 2000–2300 hrs.	158	89	79	79	74	158	199	215	148	197	138	177	167	156	136	173
6. Luminescent cells	71	68	42	21	54	141	243	892	602	993	446	640	237	118	157	52

Apr.			May				June			July				Aug.			Sept.			Oct.
12	20	27	4	10	17	31	7	16	21	28	5	13	16	27	3	13	30	7	13	28	11
1. 179	94	150	96	112	152	136	66	65	8	31	19	50	2	10	41	25	34	83	217	199	137
2. 196	117	208	107	152	205	164	84	89	18	40	26	78	5	17	87	41	45	104	260	206	177
3. 78	78	115	84	93	119	81	49	44	1	10	15	32	0	5	12	3	8	62	201	189	90
4. 175	98	180	98	135	173	—	70	66	—	34	18	38	3	16	67	33	—	85	196	199	—
5. 194	93	124	98	97	—	157	64	66	—	40	22	29	—	9	18	7	42	89	250	—	158
6. 70	23	35	26	51	11	45	13	12	1	2	8	40	0	40	172	160	292	20	33	29	54

Variation of Luminescence in Water Samples

Fifteen-liter surface water samples were brought into the laboratory darkroom from near the harbor dock of the Woods Hole Oceanographic Institution. Dates of sampling are shown in Figure 1 and are given in Kelly (1968); luminescence stimulated by bubbling air through the water was measured about every week throughout the year. The method and equipment were described by Kelly and Katona (1966). All measurements were performed in a darkroom at sea-surface temperature. For consistency the equipment and procedures were carefully kept the same throughout the entire program. Samples were collected after dark the day before measurements commenced around midday. Measurements were taken every hour to detect any endogenous diurnal rhythms that might have been present (see Kelly and Katona, 1966). Observations were also made on the effects of light inhibition of luminescence. They are described below.

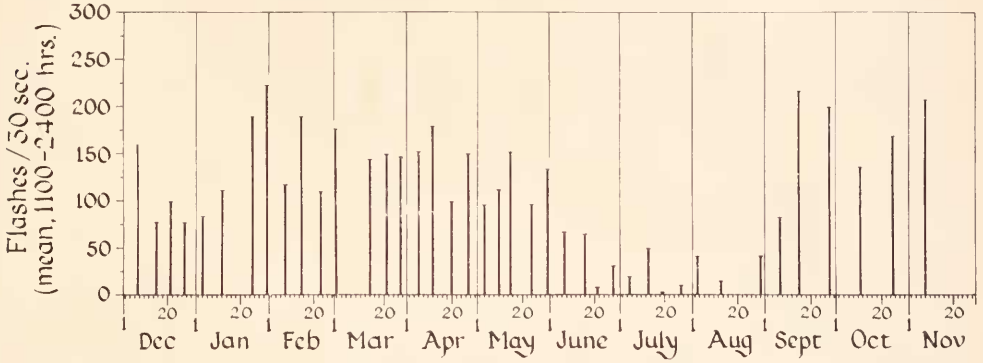


Figure 1. Bioluminescence recorded in water samples from Woods Hole Harbor during 1965 and 1966.

The amounts of luminescence found throughout the year are shown in Table II and Figure 1. These are compared below to the numbers of luminescent cells found in the water. The detailed measurements were too numerous to report here but are given by Kelly (1968). Surprisingly no significant endogenous rhythms were found. As shown in Table II, the averages of the flashing rates during midday and after dark were similar: 139.6 and 136.8 flashes per 30 seconds, respectively, through the end of May. These averages suggest a lack of any significant endogenous day-night rhythms in flashing. There was usually some variation within any day's observations as shown in the examples in Figure 2. However, the variation was usually random, and it has been impossible to determine any cause for it. There were increases in flashing rates after dark on the 12th and 25th of January and the 8th and 23rd of February, but these were probably fortuitous since they were not repeatable. Figure 2 shows typical daily measurements from November through May plotted against time of day. The curve marked by ▲ (from Kelly and Katona, 1966) is typical of the endogenous rhythm found in August and September, 1965. The one marked by △ shows the measurements of January 12, 1966, which were the most suggestive of an endogenous rhythm during 1966. The one shown by ○, taken January 25, 1966, is more typical and

shows no rhythm. The last curve, ●, is a plot of the means and its lack of variation indicates that hourly variations on any given date were random.

The endogenous rhythms found by Kelly and Katona (1966) in August, 1965, were thus not present during the rest of the year. The genus *Gonyaulax* was dominant during the August, 1965, studies, but luminescent forms of the genus were not often found during the rest of the year. Also, *Gonyaulax* is the only genus reported to have an endogenous luminescence rhythm in culture. It would

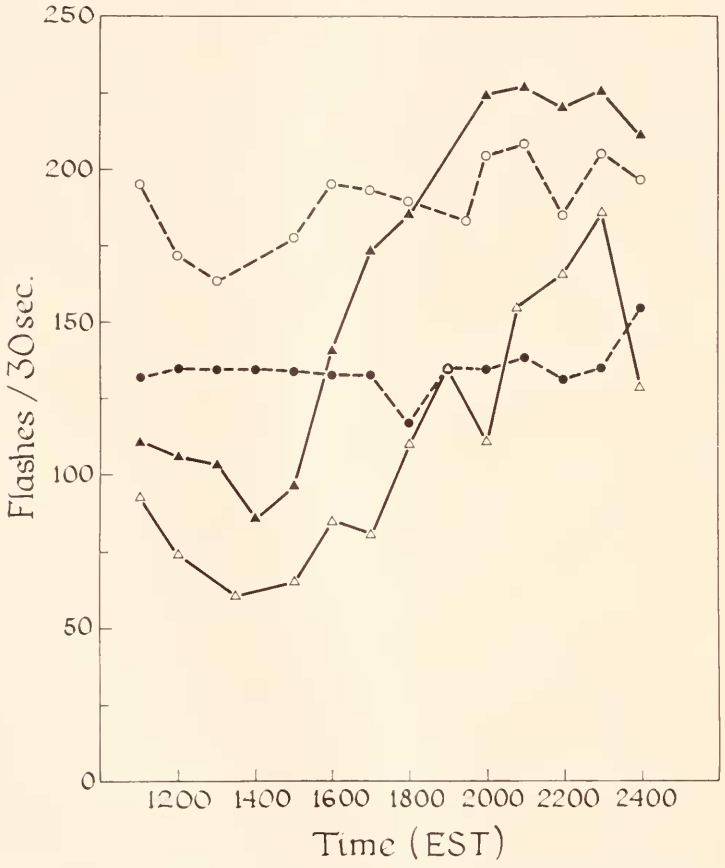
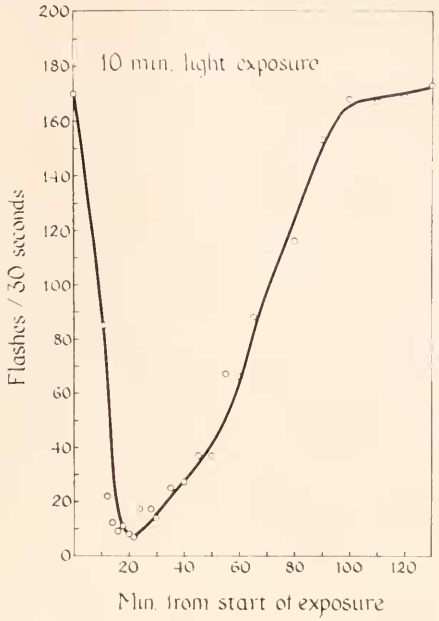
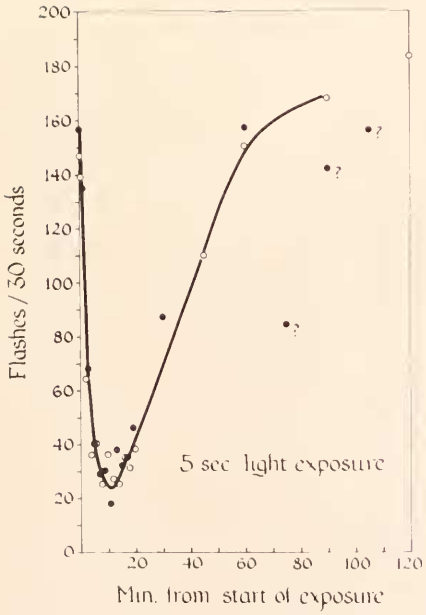


FIGURE 2. Luminescent flashing in samples of water from Woods Hole Harbor measured from noon to midnight on various dates. See text for explanation.

appear therefore that endogenous diurnal rhythms of luminescence are uncommon insofar as the situation in Woods Hole is typical.

Numerous observations were made that indicated dinoflagellate flashing is nearly always inhibited by light. This would explain the daytime decreases in luminescence usually recorded in the natural environment (Clarke and Kelly, 1965; Kelly and Katona, 1966). Kelly and Katona (1966) found light inhibition of flashing as well as an endogenous diurnal rhythm in sensitivity to that inhibition





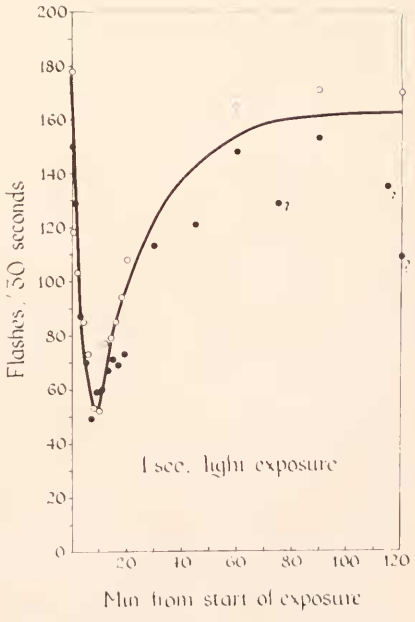


FIGURE 3. Light inhibition of luminescence in water collected from Woods Hole Harbor. See text for details.

during August, 1965. In the subsequent work casual observations of flashing inhibition were made at least once a month by turning on the lights in the darkroom (350 lux at the water surface, G.E. "cool white" bulbs, approximately 4100° K color temperature) at the end of a day's observations and measuring the effect on flashing rate. Inhibition was always found, although the amount of inhibition varied greatly.

Light inhibition of luminescence was quantitatively measured on April 13, 1966, in water collected on April 11 and kept continuously in the dark. Results are shown in Figure 3. The water was exposed to the room lights (350 lux as above) for periods of 10 minutes, 5 seconds, and 1 second. All of these exposures gave considerable inhibition. In the 5-second and 1-second observations two barrels of water were used simultaneously (the white and black circles in Figure 3). Recovery was always complete within 100 minutes of the start of exposure. Interestingly, minimal flashing rates were found 10 minutes after the end of exposure in all cases, including during the 10-minute observation, *i.e.*, inhibition appears to commence when the lights go on. An explanation of this must await better understanding of the physiology of these organisms.

Variation of Dinoflagellate Populations

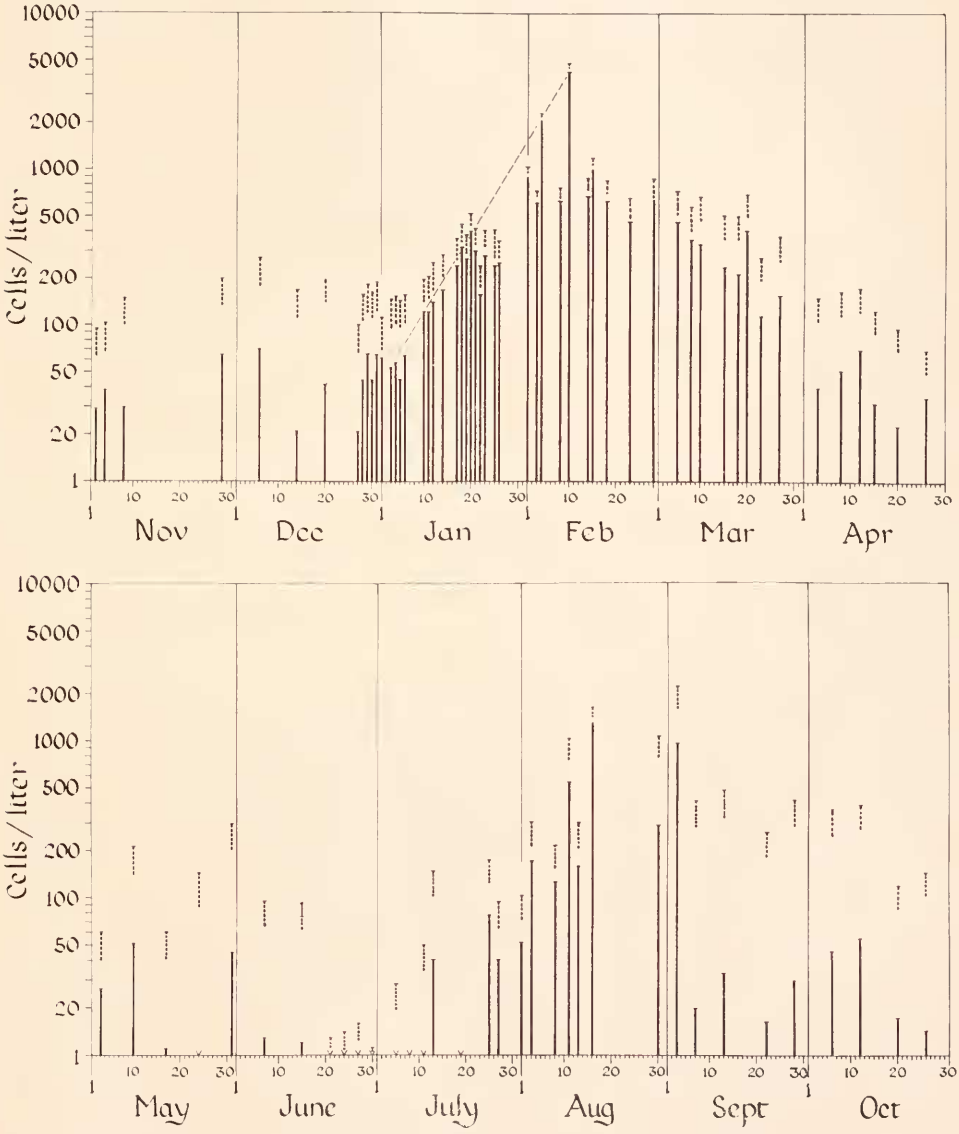
It was necessary to know the concentrations of the luminescent cells so as to determine if sufficient numbers were present to account for the recorded luminescence. Since very few cells per liter may produce measurable quantities of luminescence, and since usual methods of determining phytoplankton populations depend on small (usually 200-ml.) water samples (Braarud, 1958), it was necessary to use a new counting technique.

A quantitative 10-liter water sample was taken from the Woods Hole Oceanographic Institution dock and a surface tow was taken for taxonomic use with a 0.035-mm. mesh aperture, 20 cm. diameter net. Dates of sampling are shown in Figures 4 and 5. The 10-liter quantitative samples were passed through a 400-mesh (0.035-mm. mesh aperture) stainless steel screen. The retained material was very carefully washed from the screen with 15 to 25 ml. of Millipore-filtered sea water from a squeeze bottle and the concentrated samples were preserved with Rhode's iodine or with formalin. The samples were allowed to settle for at least 24 hours, the supernatant was removed, the residue was transferred with washing into a 10-ml. graduated conical centrifuge tube, and the samples were again allowed to settle. The supernatant was again removed, the sample was transferred to a watchglass, and the centrifuge tube was rinsed with sufficient water to bring the sample volume to 1 ml. The sample was strongly agitated in the watch glass and a 0.1-ml. aliquot was transferred to a microscope slide using a hypodermic syringe. The syringe was rotated continuously to prevent the organisms from settling in the barrel during transfer. The dinoflagellates on the slide were tabulated and counted by species under the 10-power objective of a compound microscope. A second or third 0.1-ml. aliquot was examined if necessary to count enough organisms. Since the organisms in 10 liters were concentrated into 1 ml., 0.1 ml. of the concentrate contained the organisms in 1 liter of the original sea water.

Specimens from the net tows were drawn and identified. The taxonomy is discussed by Kelly (1968) and will be published elsewhere. Although immediate

sight identification of dinoflagellates is difficult, it was possible to recognize the species in a sample during counting if detailed studies of the two samples were made first.

There were possible sources of error in the sampling technique but with care all proved negligible. Sample losses during transfer and during removal of the super-



FIGURES 4 and 5. Dinoflagellate concentrations found in samples of water from Woods Hole Harbor during 1965 and 1966. Dashed lines: total dinoflagellates. Solid lines: luminescent species. The straight dashed line points out an apparent logarithmic increase in populations that were nearly all *Peridinium subinerme*.

natant were not significant since examination of the supernatants, glassware, and screen very rarely showed specimens left behind. Inconsistencies due to transfer to the slide and measurement of the sample aliquots were negligible since results were repeatable (see Table III). Similarly, these repeatable results argue against inconsistencies in the original sampling and screening. Some cells may have passed through the screen; indeed, Wheldon (1939) found considerable loss of specimens when collecting on 0.065-mm. aperture mesh bolting silk. However, many species are smaller than 0.065 mm., but few are smaller than the 0.035-mm. aperture mesh used here. Since no armored dinoflagellates could be found on

TABLE III

Dinoflagellate concentrations in cells per liter measured at various times of day in Woods Hole Harbor on November 29, 1965

Species	Time			
	1530	1730	2100	2300
Ceratium fusus	10	8	9	8
C. lineatum	77	78	74	84
C. macroceros	4	5	5	5
C. tripos	38	36	39	28
Glenodinium lenticula	13	17	17	23
Gonyaulax spp.	1	1	1	1
Peridinium claudicans	3	6	6	4
P. divergens	3	3	2	2
P. leonis	3	3	2	2
P. oceanicum	2	2	1	1
P. ovatum	6	5	4	6
P. pallidum	37	36	33	37
P. subinerme	2	2	3	3
Phalacroma spp.	1	1	1	1
Total of all species	199	203	196	204
Total of luminescent species	64	66	61	63
Liters counted	2	2	2	2

cleared Millipore filters through which screened water was passed, and since water passed through the screen rarely showed bioluminescence, loss through the screen must have been usually insignificant.

Possible sources of error are misidentification of species of small size or confusing shape and normal random sampling errors. Misidentification was rare, and was insignificant in the overall results. Extra aliquots were counted so as to increase the sample size when the specimens were few enough to give significant random counting errors.

The concentrations of total armored dinoflagellates and of luminescent dinoflagellates are shown in Table II and in Figures 4 and 5. Thirty-two species were present of which 16 were luminescent. The species are listed in Table I; their concentrations are given in Kelly (1968). Maximal numbers of dinoflagellates occurred during January, February, and March, and during August and September, with decreases in between. The concentrations are compared below with the

amounts of bioluminescence recorded. Variations in cell concentrations during a single day (November 29, 1965) are given in Table III. The uniformity of these results shows a lack of tidal and diurnal fluctuation in populations in Woods Hole Harbor and verifies the reproducibility in the counting method.

Correlation of Luminescence with Dinoflagellate Concentrations

The regression lines of luminescent dinoflagellate concentrations and flashes recorded per 30 seconds were calculated and are plotted in Figure 6.

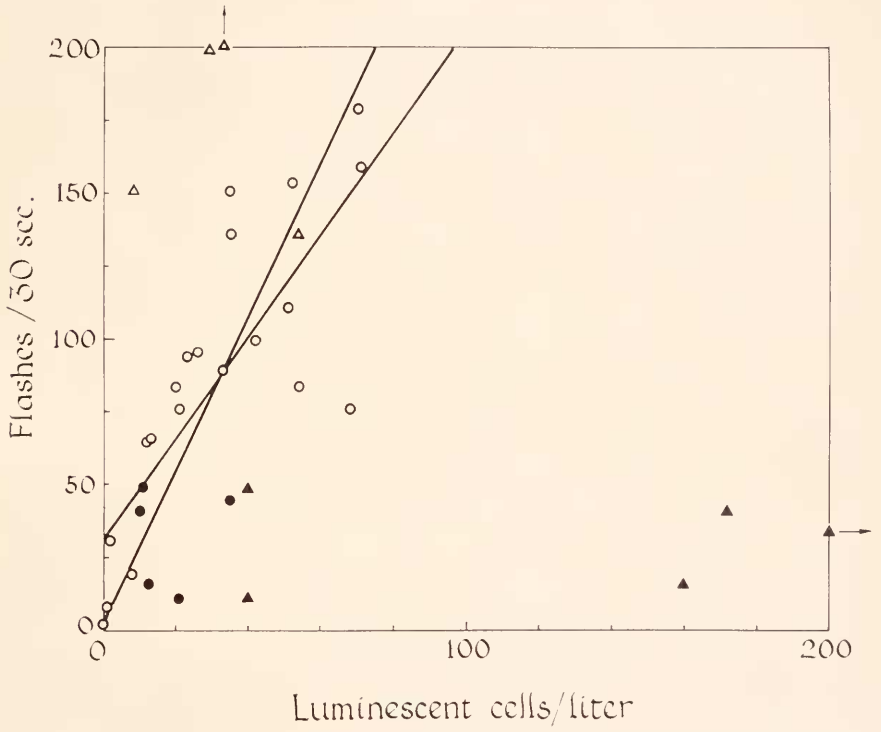


Figure 6. Correlations of dinoflagellate concentrations and luminescence in samples from Woods Hole Harbor during 1965 and 1966. See text for details.

On a few occasions described below the data were obviously invalid and not included in the calculations. The high flashing rates on May 17 and between September 13 and October 11 were due to luminescent cells too small to be collected and counted and hence were deleted from the correlations. They are designated by △ in Figure 6. The luminescence of the small cells was realized because luminescence was recorded in water that passed through the collecting screen. Flashing in the screened water varied from 53 to 103 flashes per 30 seconds compared to 136 to 217 flashes per 30 seconds in the unscreened water. However, the flashing rates in the screened water were probably not closely related to the number of luminescent small cells originally present since some may have been retained on

the screen and many that passed through were probably damaged. A liter of water collected on September 15 was passed through the screen and centrifuged. Many diverse microflagellates were collected, but it was impossible to determine which were responsible for the luminescence.

The flashing rates from July 13 and from July 27 through August 30 (designated by ▲) were low for the number of apparently luminescent cells counted. It was at first feared this discrepancy might be due to some failure in technique such as contamination of the samples with toxic materials, but visual observation while swimming off the dock at night showed qualitatively less luminescence than would have been expected from the populations of *Gonyaulax* spp. counted in the samples. If flashing rates for those dates are plotted against luminescent cell counts not including *Gonyaulax* spp. they fall within the expected range (● in Figure 6). The data from those days were not used in the regression calculations and it seems likely that the *Gonyaulax* spp. populations were not luminescent. This was realized after the populations had declined, too late to directly test the luminescence of the cells.

As the number of flashes increased they tended to overlap on the records and thus at higher flashing rates many of the flashes could not be counted. Those luminescence records could not be expected to have a direct relationship with the number of luminescent cells present. Because of this, samples with more than 100 cells per liter were not included in the regression calculations.

The equations of the regression lines shown in Figure 6 are as follows:

$$Y = (1.75 \pm 0.88)X + (31.5 \pm 21.2) \quad (1)$$

$$X = (0.38 \pm 0.68)Y + (-0.5 \pm 35.5) \quad (2)$$

X and Y represent luminescent cells per liter and mean flashes per 30 seconds, respectively. The errors are given for a 90% confidence interval. The calculations show a correlation coefficient of 0.81 between flashing rates and cell concentration, which with this sample size assures that there is less than a 0.001 probability that there is no correlation.

It is impossible to give a simple ratio relationship between luminescent dinoflagellate population density and flashing rates because of the scatter in the regression curves. This scatter indicates that factors other than simple population density influenced the amount of flashing recorded, which is not surprising considering the diverse environmental conditions and species studied. Species may differ in the number of cells capable of luminescence. For example, not all specimens of *Ceratium fusus* were capable of flashing, while nearly all of *Peridinium subinerme* were. Similarly a particular species may differ in its ability to luminesce under different environmental conditions, *i.e.,* the ability may be influenced by available energy, by nutrient concentrations, or by temperature. Also, different species or a particular species under different environmental conditions might differ in threshold to stimulus, and hence the constant stimulus used in the experiments might more readily produce flashing in some populations than in others.

It is nonetheless apparent that there was a strong relationship between the amount of flashing and the density of the luminescent populations present in the water. Except in the few instances when small protozoa were mainly responsible

for the luminescence, armored dinoflagellates were present in sufficient numbers to explain the flashing recorded.

Discussion

From this work it may be concluded that for the period studied dinoflagellates were the only major cause of bioluminescence in Woods Hole Harbor, except on rare occasions when microflagellates were present that were too small to be taken on the collecting screen. The question arises whether the armored dinoflagellates are able to account for the luminescence that is found almost everywhere in the surface regions of the oceans. Extensive studies at other locations (Kelly, 1968; to be reported separately) indicate that dinoflagellates are responsible for luminescence in many diverse oceanic environments. Dinoflagellates are common organisms (Lebour, 1925; Schiller, 1937) and are evidently commonly luminescent.

The question also arises whether dinoflagellate luminescence has evolved separately in several diverse dinoflagellate genera, or whether the luminescent species are derived from a common ancestor. Because of the diverse biochemical systems of luminescence in other marine organisms such as euphausids, ostracods, shrimp, and bacteria (Harvey, 1952; Johnson and Haneda, 1966) it seems likely that bioluminescence evolved several times in the oceanic environment. This argues a strong selective pressure for bioluminescence in the sea. Several features of dinoflagellate luminescence, however, argue that the systems are biochemically and hence genetically related.

The four species whose spectral outputs have been studied have similar emission curves (Fig. 7). *Pyrodinium bahamense, Gonyaulax polyedra,* and *Pyrocystis lunula,* very diverse species, all have a spectral peak between 470 and 480 nM., rising sharply from between 450 and 435 nM. and dropping off by 550 nM. (Taylor *et al.,* 1966; Hastings and Sweeney, 1958; Swift and Taylor, 1967). *Noctiluca scintillans* has a similar light output but its spectrum was not as accurately measured (Nicol, 1958). The spectral differences between these species are very slight and can probably be attributed to differences in technique. Although the emission curves of other organisms peak near this wavelength, they usually have a different shape (Johnson and Haneda, 1966).

The very similar emission spectra of widely diverse dinoflagellate species argue for the same light-producing enzyme-substrate reaction throughout the group since the bonding energies and structure of the light-producing molecules determine their output spectra. In addition, Hastings and Bode (1961) found that cross-reactions of *Gonyaulax monilata* and *G. polyedra* luciferin and luciferase produced light, as did cross-reactions between *G. polyedra* and *N. scintillans* (Hastings, personal communication).

Dinoflagellate luminescence spectra are similar to sea-water light transmission curves and this suggests an adaptation of luminescent dinoflagellates to the environment: an evolutionary adaptation on the molecular level. Light transmission of most offshore sea water is at a maximum between 470 and 480 nM., *i.e.,* daylight at depths below about 10 or 20 M. is blue (Jerlov and Koczy, 1951). The luminescence and maximal visual sensitivity of other marine organisms are usually in a similar color range (Nicol, 1958) although the biochemical mechanisms of their luminescence certainly differ. Although McElroy and Seliger (1962) suggest a

purely biochemical function, these spectral similarities seem to suggest an adaptation of the luminescence of marine organisms to the blue maximum transparency of sea water or to the blue sensitivity of marine animals' eyes.

The question is thus raised as to whether the identical emission spectra of dinoflagellates are due to inheritance without change from a highly adapted parent species, or whether the spectra arose independently through parallel adaptation to

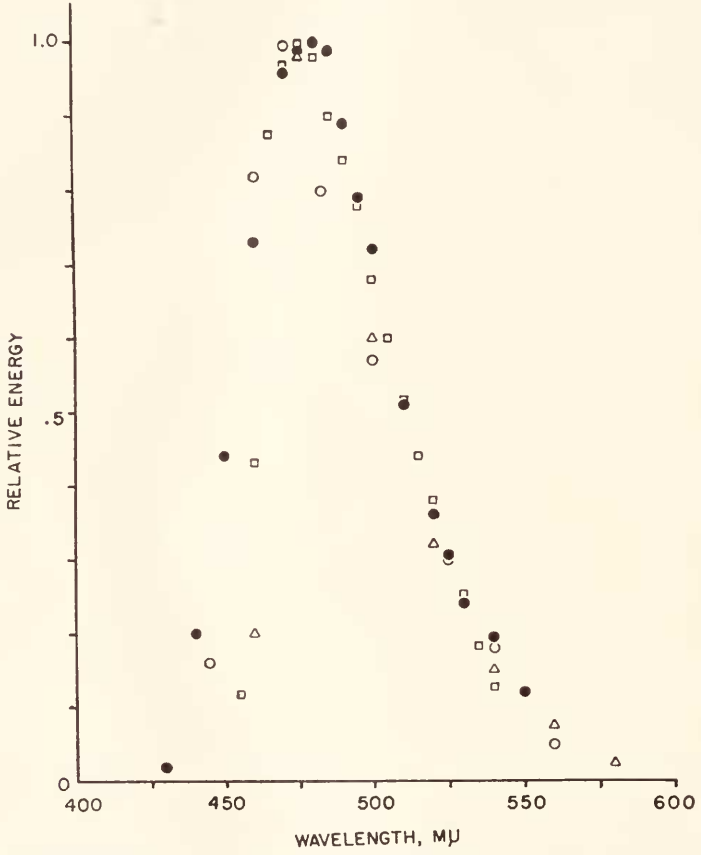


FIGURE 7. Emission spectra of luminescent dinoflagellates. ○ *Noctiluca scintillans* (Nicol, 1958); □ *Pyrodinium bahamense* (Taylor *et al.*, 1966); ● *Gonyaulax polyedra* acetone extract (Hastings and Sweeney, 1957); △ *Pyrocystis lunula* "cysts" (Swift and Taylor, 1967).

identical environmental characteristics. The cross-reactions between the species and the fact that the spectra of these species show more similarity to each other than to unrelated marine forms suggest very similar or identical chemical structures and derivation from a single parent stock. This may well have been a primitive gymnodinioid form similar to the swarm cells of *Noctiluca scintillans*. The retention by these forms of the genetic information allowing them to luminesce at the expense of energy utilization indicates that luminescence probably continues to serve some adaptive function. Further study of luminescence may have con-

siderable bearing on our understanding of both the phylogeny of dinoflagellates and the adaptive values of luminescence.

If the ecology of luminescence in the ocean is to be understood, we must be able to predict the spatial and temporal distribution of dinoflagellates and must understand their luminescence characteristics. This means that much more work is needed on the trophic dynamics of phytoplankton in the ocean, on the biogeography of dinoflagellates, on their taxonomy, and particularly on their characteristics in culture. To date only a very few luminescent species have been cultured, and the only common colorless species included in these is *Noctiluca scintillans*. It thus appears that an understanding of the characteristics and distribution of luminescence must await better understanding of other fields of marine biology.

Because of the diverse species of luminescent dinoflagellates and because of their diverse characteristics and adaptations, I greatly doubt that any single model will serve to predict or describe the distribution of luminescence. Rather, the problem will require considerable descriptive understanding of the variation of species distributions, of photoinhibition of luminescence, of endogenous rhythms, of the luminescence abilities of different species, of the energy sources for those species and of the relationships between their distribution and the environment. Such an understanding will be slow in coming and will depend on a number of diverse fields of study. I believe, however, that the present work shows dinoflagellate luminescence to be very common and to deserve attention from workers in many fields.

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Summary

1. Bioluminescence in the water in Woods Hole was measured once a week from November, 1965, to November, 1966, and was related to the occurrence of armored luminescent dinoflagellates.

2. Thirty-two species of dinoflagellates were identified, 23 of these were tested for luminescence as isolated cells. Sixteen species were found to be luminescent.

3. Population densities of dinoflagellate species were measured by counting individuals screened from water samples. The concentrations of luminescent species varied from nearly 1000 cells per liter in February, 1966, to less than 5 cells per liter during June and July, while total dinoflagellates ranged from more than 4000 cells per liter to 5 cells per liter.

4. Amounts of luminescence radiometrically recorded in air-bubble-stimulated water samples correlated with the numbers of luminescent armored dinoflagellates present. Exceptions to this correlation were found when population densities were too high for the radiometric recording techniques to be accurate and during May and August, 1966, when the luminescent organisms were too small to be collected and counted.

5. Endogenous diurnal rhythms of flashing were found only during August, 1965 (Kelly and Katona, 1966).

6. Light inhibition of flashing was found whenever it was tested (at least once each month), although sensitivity to light inhibition varied greatly—as little as 1 second of exposure to 350 lux illumination reduced flashing by $\frac{2}{3}$.

7. The ecology and evolution of luminescent dinoflagellates are discussed. It is suggested that dinoflagellates are responsible for most near-surface luminescence, that luminescent forms are derived from a single common ancestor, that the ability serves an adaptive function, as yet undetermined, and that much more work on a number of areas will be required before a detailed ecological understanding of dinoflagellate luminescence is obtained.

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